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An Investigation Conducted by
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BIODEGRADATION OF HYDROCARBON CONTAMINANTS BY PATUXENT RIVER SOIL MICROBIAL COMMUNITIES

Abstract This study attempted to determine the rates of aerobic biodegradation of common petroleum hydrocarbon compounds, and effects of hydrocarbon and nutrient concentrations on those rates and adaptation times. Tests were conducted by adding C-labeled compounds to jet fuel-contaminated soil from the fuel farm at the Patuxent River Naval Air Station, Maryland. Results indicated slow aerobic degradation rates for aliphatic hydrocarbon compounds, which were not appreciably enhanced by adding mineral nutrients or readily degradable organic compounds. Reasons for the slow degradation rates, in the presence of high hydrocarbon degrading microbial populations, were hypothesized but not completely determined.

NAVAL CIVIL ENGINEERING LABORATORY PORT HUENEME CALIFORNIA 93043-5003

METRIC CONVERSION FACTORS

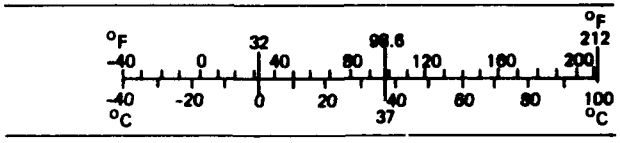
Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
		LENGTH		
in	inches	*2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
		AREA		
in^2	square inches	6.5	square centimeters	cm^2
ft^2	square feet	0.09	square meters	m^2
yd^2	square yards	0.8	square meters	m^2
mi^2	square miles	2.6	square kilometers	km^2
	acres	0.4	hectares	ha
		MASS (weight)		
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2,000 lb)	0.9	tonnes	t
		VOLUME		
tsp	teaspoons	5	milliliters	ml
Tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft^3	cubic feet	0.03	cubic meters	m^3
yd^3	cubic yards	0.76	cubic meters	m^3
		TEMPERATURE (exact)		
°F	Fahrenheit temperature	5/9 (after subtracting 32)	Celsius temperature	°C

*1 in = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.

Approximate Conversions from Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
		LENGTH		
mm	millimeters	0.04	inches	in
cm	centimeters	0.4	inches	in
m	meters	3.3	feet	ft
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
		AREA		
cm^2	square centimeters	0.16	square inches	in^2
m^2	square meters	1.2	square yards	yd^2
km^2	square kilometers	0.4	square miles	mi^2
ha	hectares (10,000 m^2)	2.5	acres	
		MASS (weight)		
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	tonnes (1,000 kg)	1.1	short tons	
		VOLUME		
ml	milliliters	0.03	fluid ounces	fl oz
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m^3	cubic meters	35	cubic feet	ft^3
m^3	cubic meters	1.3	cubic yards	yd^3
		TEMPERATURE (exact)		
°C	Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F



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13. ABSTRACT *(Maximum 200 words)*

This study attempted to determine the rates of aerobic biodegradation of common petroleum hydrocarbon compounds, and effects of hydrocarbon and nutrient concentrations on those rates and adaptation times. Tests were conducted by adding C-labeled compounds to jet fuel-contaminated soil from the fuel farm at the Patuxent River Naval Air Station, Maryland. Results indicated slow aerobic degradation rates for aliphatic hydrocarbon compounds, which were not appreciably enhanced by adding mineral nutrients or readily degradable organic compounds. Reasons for the slow degradation rates, in the presence of high hydrocarbon degrading microbial populations, were hypothesized but not completely determined.

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Biodegradation of Recalcitrant Components of Hydrocarbon Fuels by Aquifer Microbial Communities from the Patuxent River Air Station.

by

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RATIONALE

Clean, potable water sources are valuable commodities. Ground water is the largest potential source of potable water. It makes up greater than ninety-nine percent of the world's liquid freshwater (Freeze and Cherry, 1979). About fifty percent of the United States' drinking water comes from ground water (Josephson, 1980; Bitton and Gerba, 1984). Recently, ground water contamination has become a topic of concern (Page, 1981; Bitton and Gerba, 1984). Investigations conducted during the past decade have shown that ground water supplies are susceptible to both biological and chemical pollution. In many instances, at sites where ground water pollution exists, more than a single pollutant is present. Major groups of chemical ground water pollutants are petroleum products, volatile organics (especially chlorinated solvents), and pesticides. Petroleum itself is a mixture of compounds (Sachanen, 1954; Atlas, 1984; King, 1988); however, it has been recognized that mixtures of pollutants of other types have often been detected (Westrick, 1990).

Extensive research into the degradation of pollutants in ground water environments has been conducted. Subsurface microbial communities have been demonstrated to have the ability to degrade a range of natural and xenobiotic compounds (Long, 1986; Aelion et al., 1987; Lee et al., 1988; Swindoll et al., 1988). Much of this work has focused on the degradation of individual compounds (Boething and Alexander, 1979; Larson and Ventullo, 1983; Aelion et al., 1987; Kuhn et al., 1988; Swindoll et al., 1988, Dobbins, 1989). Researchers studying the degradation of mixtures consisting of oil and petroleum pollution also focused on the degradation of individual components of concern, the aromatics in particular (Wilson and Rees, 1985; Wilson et al., 1986). Hutchins et al. (1984) looked at the degradation of a mixture of six wastewater organic compounds in soil columns. The compounds they studied were o-phenylphenol, 2-(methylthio)benzothiazole, p-dichlorobenzene, benzophenone, 2-methylnaphthalene, and p-(1,1,3,3-tetramethyl-butyl)phenol. The focus of the research was, however, the individual compounds.

Attempts to remove organics from ground water systems via ground water recovery and other physical or chemical methods have met with marginal success (McKee et al., 1972; Jamison et al., 1975; Wilson and Conrad, 1984; Hoag and Marley 1986). One method with potential for complete destruction of petroleum compounds to innocuous compounds like CO_2 and H_2O and little additional disruption of the environment is *in situ* biodegradation (Alexander, 1981; Lee et al., 1988). *In situ* bioremediation is an environmentally acceptable technique. Even though the relationship between environmental factors (concentration of pollutants, mixtures of pollutants, availability of inorganic nutrients, availability of dissolved organic carbon, oxygen, and other physical factors) and the indigenous microbial degradation rates is not yet

clearly understood (Wilson et al., 1986a; Swindoll et al., 1988a), *in situ* bioremediation has been used by many environmental engineering firms to clean up gasoline spills. Most case studies of *in situ* remediation of gasoline and petroleum focused on the aromatic fraction (BTEX) because they are hazardous compounds regulated by the U.S. Environmental Protection Agency. Implementation of *in situ* bioremediation to date seems to be limited to gasoline and petroleum spills (Lee et al., 1988). The hope is that knowledge of how mixtures are degraded and what environmental factors can impact the process will enable the design of more generic, less site-specific, and therefore more efficient and less expensive bioremediation methods.

It is well known that petroleum hydrocarbons represent a common contaminant of many aquifers. It is also well documented that microbial communities readily biodegrade both the aromatic and simple aliphatic portions of petroleum (see Atlas, 1984). Other fractions of petroleum hydrocarbons appear to be more resistent to microbial attack, particularly the cyclic aliphatic, branched aliphatic, and multi-ring polynuclear aromatic portions. As a petroleum spill or underground release ages, the more degradable fractions will disappear and the resistant materials will remain.

OBJECTIVES

The initial objective of this effort was to examine the biological transformation of cyclic and branched-chain aliphatic in aquifer solids from an aged hydrocarbon fuel spill area. The study was planned as a multi-year effort that would:

1. Characterize the rates and kinetics of aerobic biodegradation of petroleum derived hydrocarbons in samples from the Patuxent River Air Station site. Determine how the rates and kinetics are influenced by concentration of petroleum hydrocarbons.

2. Establish whether adaptation to the biodegradation of the petroleum is needed and how long it takes for which type components of the hydrocarbons.

3. Assess the impact of added inorganic and organic nutrients on the rates of aerobic biodegradation of hydrocarbons in the aquifer.

Our efforts during the first year of the project were directed toward examination of the communities' metabolic abilities, size and response to petroleum hydrocarbons. Specific questions related to cyclic and branched chain aliphatics were address only briefly at the end of the first year and were to be the main focus of subsequent years.

METHODS

Subsurface materials and sampling

Subsurface soil samples were from several sites at the Patuxent River Fuel Storage (PR) area. Two samples were recieved. A core sample from the 6-8 ft depth interval of a petroleum contaminated area was received. A second sample which was a composite of drilling tailings from Navy monitoring well 24 was also obtained. This well is in an area of aged contaminants. In each case the samples were collected by others and we had little control over the nature and history of the sample materials. The samples were stored at 5°C until they were analyzed. Samples were also obtained from a hydrocarbon spill site near Charleston, South Carolina, and has been included in some of the community assays for comparison purposes. Future work would include a metabolic characterization of the communities from the South Carolina site.

Microbial Activity Assessment

An initial soil slurry of approximately 1 gram soil (dry weight) per 10 ml water was prepared for use in all degradation assessments. This approach allows for dilution of background levels of pollutants so that the compounds added to the microcosms will predominate.

Time course experiments were conducted using procedures similar to Aelion et al. (1987). These experiments used two concentrations of radiolabeled target compound, one approximately 10 to 100 ng per gram soil (dry weight) and one approximately 10 to 100 µg per gram soil (dry weight). It is important to test the effect of target compound concentration on the extent of biodegradation because it has been demonstrated that different test compound concentrations can yield different metabolic responses (Boething and Alexander, 1979). A 10 ml aliquot of soil slurry was pipetted into 25 ml vials. Dead controls were created by adding sodium azide (a metabolic inhibitor) to a concentration of 0.5 percent by weight. Appropriate amounts of ^{14}C labeled substrate are added by pipet to each vial. The vials are then filled with sterile (autoclaved) distilled water or buffer and sealed headspace free with Teflon-lined caps. The vials are incubated inverted for an appropriate amount of time. It has been demonstrated that incubation headspace free and inverted minimizes loss of volatile substrate and CO_2 (Dobbins, 1989).

At appropriate time points, live and dead replicate sample vials are transferred to 40 ml vials using Teflon lined connector caps. The samples are then acidified to pH 2 using 20% (v/v) H_3PO_4. The vials are capped using a Teflon lined cap equipped with a bucket type center well containing 200 µl of 1N KOH. The vials are placed upright on a rotary shaker and allowed to equilibrate for 24 hours in order to trap the $^{14}CO_2$. Once the samples have been allowed to equilibrate, the base is collected using fluted filter papers and transferred to scintillation vials containing 10 ml of scintillation cocktail and then counted using a liquid scintillation counter. The percent of initially added radiolabeled substrate respired can be calculated from this data. These experiments provide information as to the length of time required for a substrate to be metabolized, identify any adaptation period required, reveal the extent of metabolism, and allow calculation of respiration rates. Estimates of cellular

utilization and incorporation of substrates into cellular material are made by using a washing of the soil materials. The steps of this method is best described using a flow chart which is presented in Figure 1. The data from these experiments provides estimates of both respiration and cellular uptake of the test substrate, allows calculation of a mass balance of added label, and allows calculation of kinetic information.

Microbial enumerations

An initial soil slurry of approximately 1 gram soil (dry weight) per 10 ml water was prepared for use in all enumeration procedures.

Acridine Orange Direct Counts

Acridine Orange direct counts (AODC) were performed on all cores. The procedure to be employed is similar to that described by Dobbins and Pfaender (1988) and Swindoll et al. (1988a). Briefly, the soil slurry is prepared using particle free water (autoclaved and filtered three times through 0.2 µm Nucleopore filters) and serially diluted with particle free water and fixed with particle free formalin to a final concentration of 2 percent. The appropriate dilutions are then applied to 0.2 µm Nucleopore filters that have been counterstained with Irgalan Black dye. The samples are then stained with a final concentration of 0.01 percent Acridine Orange. The filters are placed on a microscope slide and counted using epifluorescent microscopy. This method yields a count of the intact cells without differentiation for viability.

Plate Counts

Plate counts were conducted using the spread plate method and were plated on nutrient agar (high nutrients) and R2A agar (low nutrients) as described in Standard Methods (1989). The slurry of the subsurface material was prepared with sterile (autoclaved) distilled water and serially diluted. One milliliter of each dilution was plated, in triplicate, onto both nutrient agar and R2A plates. Plates using only dilution water served as controls. The nutrient agar and R2A plates were incubated at 20°C for 48 hours and counted. The R2A plates were then be returned to the 20°C incubator and inspected everyday until no new colonies appear. At that point the plates were recounted. Plating methods provide a count of the cells that will grow under the conditions provided.

^{14}C-Most Probable Number

^{14}C-MPNs were performed using Dobbins' (1989) modifications of the method of Somerville et al. (1985). The initial soil slurry was ten-fold serially diluted. A subsample of 1 ml of each dilution was transferred into a 5 ml glass minivial. Five replicates of each dilution level were prepared. Vials containing only dilution water serve as abiotic controls. Approximately 50 ng of ^{14}C labeled substrate

is pipetted into each minivial. The minivials are filled with sterile (autoclaved) distilled water or buffer and sealed headspace free with Teflon-lined caps. The minivials are incubated inverted for the appropriate amount of time. $^{14}CO_2$ is collected after incubation by placing the minivials into 25 ml vials containing a piece of fluted filter paper saturated with 1N KOH. One ml of the sample in the minivial is removed and replaced with 100 µl of 20% (v/v) H_3PO_4 to acidify. The 25 ml vials are then sealed with Teflon-lined caps and placed upright on a rotary shaker. The vials are allowed to equilibrate for 24 hours. Once the samples have been allowed to equilibrate, the filter papers are transferred to scintillation vials containing 10 ml of scintillation cocktail and counted using a liquid scintillation counter.

The samples are scored as positive or negative based on comparison with the abiotic controls. A vial that produces more counts than the mean plus three standard deviations of the abiotic controls is scored positive. Otherwise, it is scored negative. This method provides and estimate of the most probable number of microbes with the ability to degrade a specific radiolabeled substrate.

RESULTS AND DISCUSSION

Microbial Community Characterization

The Most-Probable-Number (MPN) results for amino acids and decane are shown in Table 1. Data for the two samples from the Patuxent River Site are compared to other sites we have examined as part of other studies and represent areas exposed to hydrocarbons (Camp LeJeune, Traverse City, and Charleston) and pristine areas (Lula). The amino acid results suggest numbers on the low side of average for the Patuxent River site compared to other locales. This parameter represents the total community capable of taking up a mixture of radiolabeled amino acids, and should reflect the active portion of the total community. The numbers of decane utilizers should indicate the potential for degradation of aliphatic hydrocarbons. For this parameter the PR site appears to have a significant community of hydrocarbon degraders, at least compared to all sites except Traverse City, MI where a long standing plume of aviation gasoline exists. This data suggested that active breakdown of the hydrocarbons should be likely.

Table 2 presents the total plate count data on both nutrient agar and R2A agar. Nutrient agar is a high carbon content agar that will generally give higher counts for communities that have experienced nutrient enrichment. R2A agar has a lower nutrient content and will give higher counts for communities that are adapted to more oligotrophic conditions. The results indicate that the numbers detected by the two media are about the same for the core sample from PR which suggests a nutrient enriched site and a potentially active community. The results for the composite sample shows slightly higher numbers in the R2A agar which may mean a less active community, or at least one better adapted to lower nutrient situations.

Metabolic Activity Assessment- Amino Acids:

We have addressed the microbial metabolism of three types of substrates in our attempt to determine how the microorganisms from the subsurface respond to the presence of petroleum contaminants. One issue of significance is the general metabolic abilities of the community. The ability to metabolize amino acids was used as a indicator of the community's general "health" and a potential predictor of their response to other materials. If the organisms are not metabolizing amino acids they are probably not able to metabolize much else. Figure 2 shows the mineralization of amino acids at 162 ug/g soil by the community from the core sample. This community responded fairly quickly to the addition of amino acids with approximately 35% being mineralized within the first 24 hours. This is consistent with the MPN results (Table 1) which indicated significant numbers of amino acid utilizers. While we do not have measurements of other possible fates it is reasonable that a significant amount is in cell biomass and most of the amino acids were totally utilized.

The incubation of the composite sample from the aged petroleum spill site (Navy 24) with a low (134 ng/gm soil) and higher (10.6 ug/gm soil) concentration of amino acids produced the results shown in Figure 3. Obviously much lower amounts of amino acid metabolism was detected. There was little difference between concentrations, with the lower level being mineralized to a slightly greater extent. This difference is probably not significant and may reflect a shift to slightly more cellular incorporation at the higher concentration. The soil we received had a pH of approximately 5.8 which raised a concern that this may be responsible for the slow metabolism observed. The results shown in Figure 4 suggests there is little difference between the mineralization results obtained at ambient pH and if the pH is adjusted to 7.0. This suggests that pH is not a major influence in the range encountered and some other factor is responsible for the lower than expected metabolism measured.

Metabolic Activity Assessment- Aliphatic Hydrocarbons:

Decane was used as substrate representitive of the aliphatic hydrocarbons. Since there appears to be little substrate specificity in the degradation of straight chain aliphatics this seemed a reasonable choice (Atlas, 1984). Mineralization of decane by the 6-8 ft interval core community is shown in Figure 5. The community mineralizes decane without an adaptation period but the activity reaches a plateau when only 5-15 percent of the material has been converted to CO_2. Slightly more is mineralized at the lower concentration. Comparable data for the composite sample is shown in Figure 6. In this case two separate experiments at approximately the same substrate concentration were conducted to evaluate reproducibility. No differences were noted until approximately day 10 of incubation at which time the slightly higher concentration showed a small increase. As for the core sample very little of the decane was converted to carbon dioxide with a maximum near 15% conversion.

The data from both subsurface soil suggested that decane was not readily mineralized even though the MPN results suggested the presence of a community capable of hydrocarbon metabolism. There are several possible explanations for the absence of extensive mineralization, which include:

1. The community may be present but unable to carry out the transformations because they are lacking some essential nutrient or growth factor.

2. Some period of acclimation is needed to adapt to more active degradation of the hydrocarbons or for some toxin or inhibitor to be altered.

3. Some substance(s) are present that inhibit the community's metabolism of hydrocarbons are either present in the soil or are formed during the early stages of decane metabolism.

Several experiments were conducted to examine these potential causes of the observed low metabolism. In one set of experiments, composite samples were incubated for 80 days with a layer of unlabeled decane overlaying the solution and then spiked with labeled decane at 3.1 ug/gm soil. If the community had adapted to a higher degradation rate more rapid metabolism than seen in Figure 6 should be evident. The results presented in Figure 7 demonstrate that either the community cannot adapt further, or an adaptation period longer than 80 days will be required.

To address the issue of metabolism limited by mineral nutrients samples of composite material were amended with nitrogen and phosphorus salts at a C:N:P ratio of 100:50:5 and incubated with 121 ng/gm soil of decane. The results in Figure 8 suggest that mineral nutrients are not the factor limiting degradation. In an additional experiment, composite sample material was amended with yeast extract (50 mg/l) which provides vitamins and other organic cofactors as well as a readily utilizable carbon source. The results in Table 3 show the impact of yeast extract addition on both amino acid and decane metabolism. In these experiments we looked at both mineralization, uptake into cells, and the material remaining attached to the soil. The assumption is made that the soil associated material present in the live samples at levels above what is seen in the dead controls represents biologically generated materials, cells and extracelluar materials. The amino acids results show that the production of soil associated biological materials is greater at the higher concentration (2.7 ug/gm), with respiration and cellular uptake about the same. When yeast extract is added and the samples incubated for 9 days prior to adding amino acids, the overall metabolism is the same as for the low concentration addition alone. The amount of respiration, however has doubled, at the expense of the soil bound material. If the assumption is made that soil bound material is largely cells or extracelluar polymers, then the effect of pre-incubation with yeast extract is a shift in metabolism to more respiration. Similar patterns have been noted previously for subsurface communities (Aelion et al., 1989). For decane metabolism increases in all metabolic compartments (respiration, cells, soil) with time were observed. The respiration values are similar to those shown in Figure 6. These results also show that a small fraction goes into cells, and that soil associated materials (cells and biologically generated extracellular materials) represent a fate about as important as respiration. Preincubation of these samples with yeast extract does not seem to have any stimulatory effect, in fact the reverse appears to be true. The overall results of these organic nutrient addition experiments suggest that a significant stimulation of metabolism is not achieved and that the inability of the community to significantly degrade hydrocarbons is not due to a limitation of nutrients.

Metabolic Activity Assessment- Cyclic Aliphatic Hydrocarbons:

We conducted one experiment on the biodegradation of cyclohexane added to the composite sample. The results in Figure 9 show that this community is not capable of significant breakdown of this compound.

Further investigations of the stimulation or induction of biodegradation of this compound would be the focus of further research should additional funding become available.

Interpretation:

While the results presented do not lead to any solid explanations of the processes observed some conclusions can be drawn.

1. Significant rates of aliphatic hydrocarbon metabolism were not observed in either of the samples from the aquifer beneath the Patuxent River site.

2. In both samples, there were numbers of both amino acid and hydrocarbon degraders that would be predicted to produce appreciable metabolism.

3. The addition of mineral nutrients and readily degradable organics did not result in any enhancement of the rates of aliphatic hydrocarbon degradation.

4. The above data taken together suggest that some material(s) exists in the sample matrix that inhibits the native microbial community's ability to degrade aliphatic hydrocarbons. The nature of the inhibitor(s) is not known. It is possible that some agent exists in the soil that is toxic, or that an inhibitor was formed through the metabolism of some fraction of the fuel spilled in the aquifer which results in inhibition of the degradation of the remaining fractions. It is also possible that an inhibitor is produced during the early stages of metabolism of the test chemical that inhibits further degradation. The identification of the agent(s) responsible for the inhibition should be a major priority, as should determing whether the inhibition can be overcome naturally or with amendment of the system in a manner consistent with a remediation strategy.

If funds for additional research become available an investigation of the inhibitory materials will have a high priority. Toxicity screening techniques are now available that will make the identification of the source and nature of the inhibitory materials possible.

Literature Cited

Aelion, C.M., C.M. Swindoll, and F.K. Pfaender. 1987. Adaptation to and Biodegradation of Xenobiotic Compounds by Microbial Communities from a Pristine Aquifer. Appl. Environ. Micro. 53: 2212-2217.

Aelion, C.M., D.C. Dobbins, and F.K. Pfaender. 1989. Adaptation of aquifer microbial communities to the biodegradation of xenobiotic compounds: Influence of substrate concentration and preexposure. Environ. Toxicol. Chem. 8:75-86.

Alexander, M. 1981. Biodegradation of Chemicals of Environmental Concern. Science 211: 132-138.

Atlas, R.M. (ed). 1984. Petroleum Microbiology. Macmillan Publishing Company, New York.

Barker, J.F., and G.C. Patrick. 1985. Natural Attenuation of Aromatic Hydrocarbons in a Shallow Sand Aquifer. In: Proceedings of the Petroleum Hydrocarbons and Organic Chemicals in Ground Water - Prevention, Detection, and Restoration, November 13-15, National Water Well Association and American Petroleum Institute. pp. 160-177.

Bauer, J.E., and D.C. Capone. 1985. Degradation and Mineralization of the Polycyclic Aromatic Hydrocarbons anthracene and naphthalene in Intertidal Marine Sediments. Appl. Environ. Micro. 50: 81-90.

Bitton, G., and C.P. Gerba (eds.). 1984. Groundwater Pollution Microbiology. John Wiley & Sons, Inc., New York.

Boething, R.S., and M. Alexander. 1979. Effect of Concentration of Organic Chemicals on their Biodegradation by Natural Microbial Communities. Appl. Environ. Micro. 37: 1211-1216.

Dibble, J.T., and R. Bartha. 1979. Effect of Environmental Parameters on the Biodegradation of Oil Sludge. Appl. Environ. Micro. 37: 729-739.

Dobbins, D.C. and F.K. Pfaender. 1988. Methodology for Assessing Respiration and Cellular Incorporation of Radiolabeled Substrates by Soil Microbial Communities. Microbial Ecol. 15: 257-273.

Dobbins, D.C. 1989. Variations in Biodegradation Rates of Four Different Organic Substrates and in Other Microbiological Parameters Among Subsurface Solids Samples. Doctoral Dissertation, University of North Carolina.

Dunlap, W.J., J.F. McNabb, M.R. Sccalf, and R.L. Cosby. 1977. Sampling of Organic Chemicals and Microorganisms in the Subsurface. EPA-600/2-77-176. U.S. Environmental Protection Agency, Washington, DC.

Freeze, R.A., and J.A. Cherry. 1979. Groundwater. Prentice Hall, Inc., Englewood Cliffs, New Jersey.

Hoag, G.E., and M.C. Marley. 1986. Gasoline Residual Saturation in Unsaturated Uniform Aquifer Materials. J. of Environ. Engin. 112: 586-604.

Hoeniger, J.F.M. 1985. Microbial Decomposition of Cellulose in Acidifying Lakes of South-Central Ontario. Appl. Environ. Micro. 50: 315-322.

Hutchins, S.R., M.B. Tomson, J.T. Wilson, and C.H. Ward. 1984. Microbial Removal of Wastewater Organic Compounds as a Function of Input Concentration in Soil Columns. Appl. Environ. Micro. 48: 1039-1045.

Jamison, V.W., R.L. Raymond, and J.O. Hudson. 1975. Biodegradation of High-Octane Gasoline in Groundwater. Dev. Ind. Microbiol. 16: 305-312.

Josephson, J. 1980. Safeguards for Ground Water. Env. Sci. and Technol. 14: 38-44.

King, R.W. 1988. Petroleum: Its Composition, Analysis and Processing. In Occupational Medicine: The Petroleum Industry, Vol. 3, Number 3, 409-430.

Kuhn, E.P., J. Zeyer, P. Eicher, and R.P. Schwarzenbach. 1988. Anaerobic Degradation of Alkylated Benzenes in Denitrifying Laboratory Aquifer Columns. Appl. Environ. Micro. 54: 490-496.

Larson, R.J., and R.M. Ventullo. 1983. Biodegradation Potential of Groundwater Bacteria. Proceedings of the Third National Symposium on Aquifer Restoration and Groundwater Monitoring, May 25-27. pp. 402-409.

Lee,M.D., J.M. Thomas, R.C. Borden, P.B. Bedient, C.H. Ward, and J.T. Wilson. 1988. Biorestoration of Aquifers Contaminated with Organic Compoounds. CRC Critical Reviews in Environmental Control 18: 29-89.

Long, S.C. A Descriptive Study of the Degradation of Natural and Xenobiotic Compounds by the Subsurface Microbial Community from an Aquifer in Lula, Oklahoma. Master's Thesis, University of North Carolina at Chapel Hill, 1986.

McKee, J.E., F.B. Laverty, and R.M. Hertel. 1972. Gasoline in Groundwater. J. of WPCF 44: 293-302.

Nelson, M.J.K., S.O. Montgomery, E.J. O'Neill, and P.H. Pritchard. 1986. Aerobic Metabolism of Trichloroethylene by a Bacterial Isolate. Appl. Environ. Micro. 52: 383-384.

Page, W.G. 1981. Comparison of Groundwater and Surface Water for Patterns and Levels of Contamination by Toxic Substances. Env. Sci. and Technol. 15: 1475-1481.

Sachanen, A.N. 1954. Hydrocarbons in Gasolines, Kerosenes, Gas Oils and Lubricating Oils. In Chemistry of Hydrocarbons, Vol. 1, Reinhold Publishing Corporation, New York, pp. 5-36.

Somerville, C.C., C.A. Monti, and J.C. Spain. 1985. Modification of the ^{14}C-most-probable-number Method for use with Nonpolar and Volatile Substrates. Appl. Environ. Micro. 49: 711-713.

Standard Methods for the Examination of Water and Wastewater, 17th edition. 1989. American Public Health Association. Washington, DC.

Swindoll, C.M., C.M. Aelion, D.C. Dobbins, O. Jiang, S.C. Long, and F.K. Pfaender. 1988. Aerobic Biodegradation of Natural and Xenobiotic Organic Compounds by Subsurface Microbial Communities. Environ. Tox. Chem. 7: 291-299.

Swindoll, C.M., C.M. Aelion, and F.K. Pfaender. 1988a. Influence of Inorganic and Organic Nutrients on Aerobic Biodegradation and on the Adaptation Response of Subsurface Microbial Communities. Appl. Environ. Micro. 54: 212-217.

Ward, T.E. 1985. Characterizing the Aerobic and Anaerobic Microbial Activities in Surface and Subsurface Soils. Environ. Tox. Chem. 4: 727-737.

Westrick, J.J. 1990. National Surveys of Volatile Organic Compounds in Ground and Surface Waters. In Significance and Treatment of Volatile Organic Compounds in Water Supplies. Lewis Publishers, Inc., Chelsea, Michigan. pp. 103-138.

Wilson, J.T., J.F. McNabb, D.L. Balkwill, and W.C. Ghiorse. 1983. Enumeration and Characterization of Bacteria Indigenous to a Shallow Water-Table Aquifer. Ground Water 21: 134-142.

Wilson, J.T., and S.H. Conrad. 1984. Is Physical displacement of Residual Hydrocarbons a Realistic Possibility in Aquifer Restoration? In: Proceedings of the Petroleum Hydrocarbons and Organic Chemicals in Ground Water - Prevention, Detection, and Restoration, National Water Well Association and American Petroleum Institute. pp.274-298.

Wilson, B., and J.F. Rees. 1985. Biotransformation of Gasoline Hydrocarbons in Methanogenic Aquifer Material. In: Proceedings of the Petroleum Hydrocarbons and Organic Chemicals in Ground Water - Prevention, Detection, and Restoration, November 13-15, National Water Well Association and American Petroleum Institute. pp.128-139.

Wilson, B.H., G.B. Smith, and J.F. Rees. 1986. Biotransformation of Selected Alkylbenzenes and Halogenated Aliphatic Hydrocarbons in Methanogenic Aquifer Material: A Microcosm Study. Env. Sci. and Technol. 20: 997-1002.

Wilson, J.T., L.E. Leach, M. Henson, and J.N. Jones. 1986a. In Situ Biorestoration as a Ground Water Remediation Technique. Ground Water Monitor Review 6: 56-64.

Table 1
Estimated Most-Probable-Numbers of Degraders
from Aquifer Samples
(mpn/g soil dry weight)

Compound	Site	Sample	MPN	95% Confidence Limits	
				lower	upper
Amino Acids	Patuxent River	Core	8.70E+04	2.59E+04	2.96E+05
		Composite	1.10E+05	3.37E+04	3.63E+05
	Camp Lejeune (1)	12-B	3.84E+04	1.30E+04	1.14E+05
		14	8.60E+01	2.70E+01	2.69E+02
		16	3.66E+03	1.28E+03	1.04E+04
	Charleston	MWGS20	2.71E+05	9.98E+04	7.38E+05
		MWGS22	2.44E+06	8.02E+05	7.41E+06
		MW5A	5.17E+03	1.82E+03	1.47E+04
	Lula (2)	9S10	1.80E+06	4.00E+04	5.90E+06
		9KK4	1.83E+05	5.60E+04	6.00E+05
Decane	Patuxent River	Composite	5.32E+03	1.56E+03	1.82E+04
		Composite	9.22E+02		
	Camp Lejeune (1)	12-U	2.00E+00	0.00E+00	1.60E+01
		14	0.00E+00	NA	NA
		16	0	NA	NA
	Traverse City (1)	44S1	1.79E+05	5.90E+04	5.43E+05
	Lula (1)	9MM2	4.00E+00	1.00E+00	2.00E+01
		9KK4	2.00E+00	0.00E+00	1.60E+01

(1) Data taken from Dobbins, 1989.
(2) Data taken from Long, 1986.

Table 2
Plate Counts of Bacteria from Aquifer Samples
(cfu/gram soil dry weight)

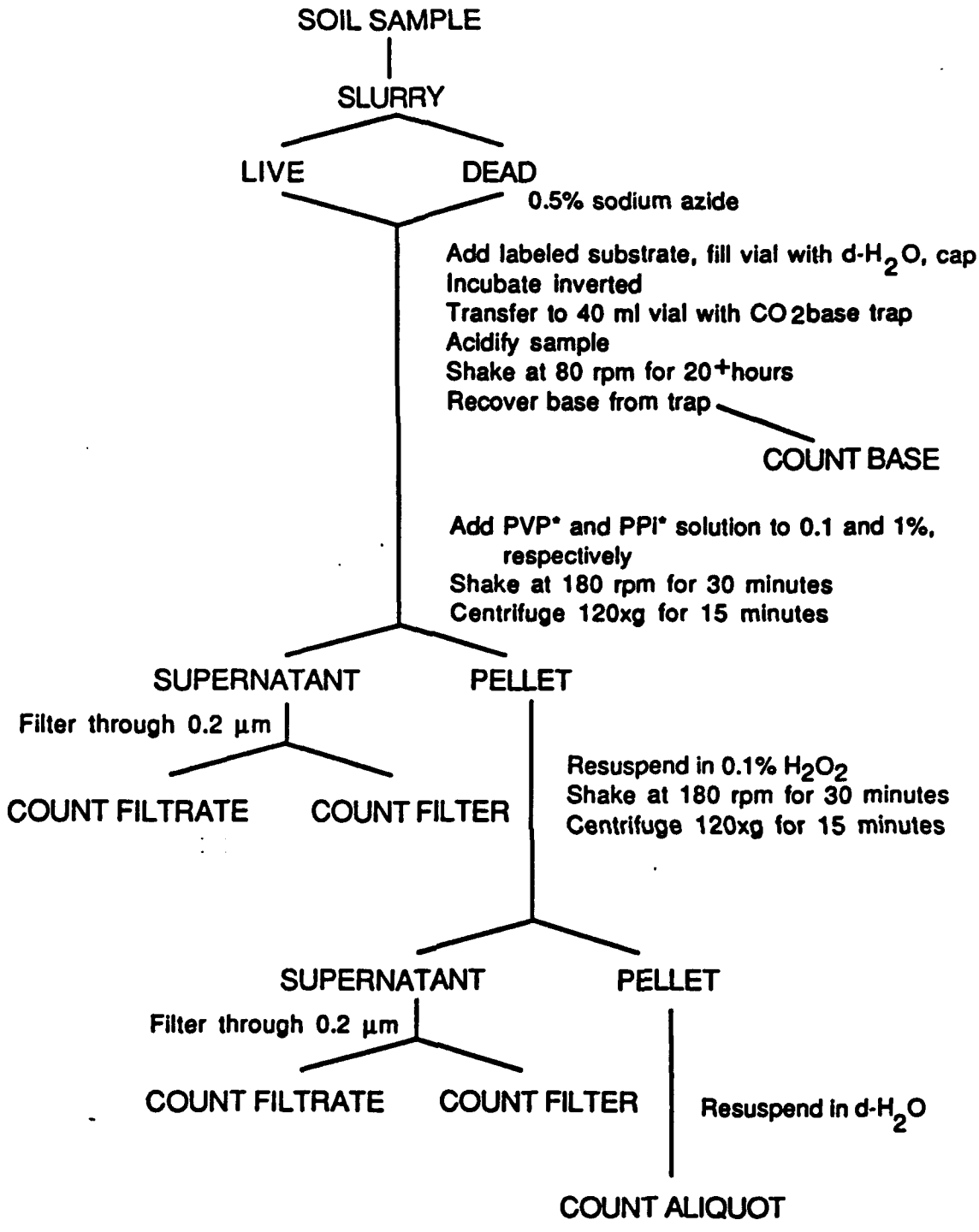
Plating media	Sample	Counts Replicates or Range		
Nutrient agar	Patuxent River (1)	3.7E+03 to1.1E+07		
	Patuxent River Core	1.01E+07	7.60E+06	
	Patuxent River Composite	9.80E+04	1.17E+05	3.60E+05
	Charleston - MWGS20	1.50E+06	1.60E+06	
	Charleston - MWGS22	3.20E+06	2.50E+06	
	Charleston - MW5A	1.30E+05	5.20E+04	
R2A Agar	Patuxent River Core	6.90E+06	9.40E+06	
	Patuxent River Composite	5.35E+05	5.23E+05	7.40E+05

(1) Data taken from Naval Civil Engineering Laboratory Report 1989.

Table 3
Metabolism Mass Balance Data
Patuxent River Composite Sample

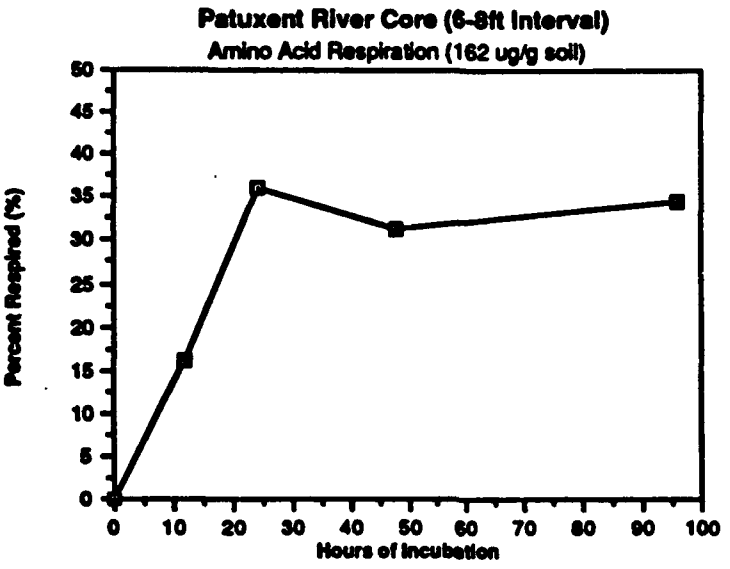
Compound	Treatment		% Respired	% Cell Uptake	% On Soil	% Metabolized
Amino Acids	2.7ug/g, pH 7	Live	13.9	7.5	43.5	61.5
	72 hour incubation	Dead	0.3	2.3	0.8	
	1.4 ug/g, pH 5.7	Live	10.2	10.1	25.8	43.7
	72 hour incubation	Dead	0.3	0.9	1.2	
	soil grown on 50 mg/l	Live	20.6	12.6	12.9	43.6
	yeast extract for 9 days	Dead	0.5	1.3	0.7	
	0.94 ug/g, pH 5					
	48 hour incubation					
Decane	2.15 ug/g, pH 7	Live	7.6	3.8	14.5	13.9
	7 day incubation	Dead	0.6	2.4	9.0	
	2.15 ug/g, pH 7	Live	14.5	4.2	23.6	26.9
	14 day incubation	Dead	1.0	3	11.4	
	soil grown on 50 mg/l	Live	0.4*	4.5	12.2	5.2
	yeast extract for 9 days	Dead	0.3	3.7	7.9	
	0.97 ug/g, pH 5					
	14 day incubation					

*CO2 recovery efficiency was inconsistent in this experiment



* PVP - polyvinyl pyrrolidone
* PPi - sodium pyrophosphate

Figure 1. Degradation Experiment Procedure



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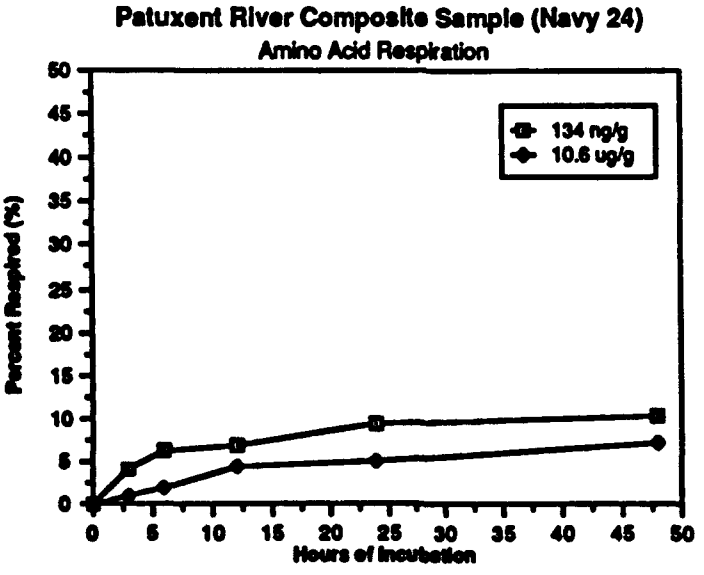

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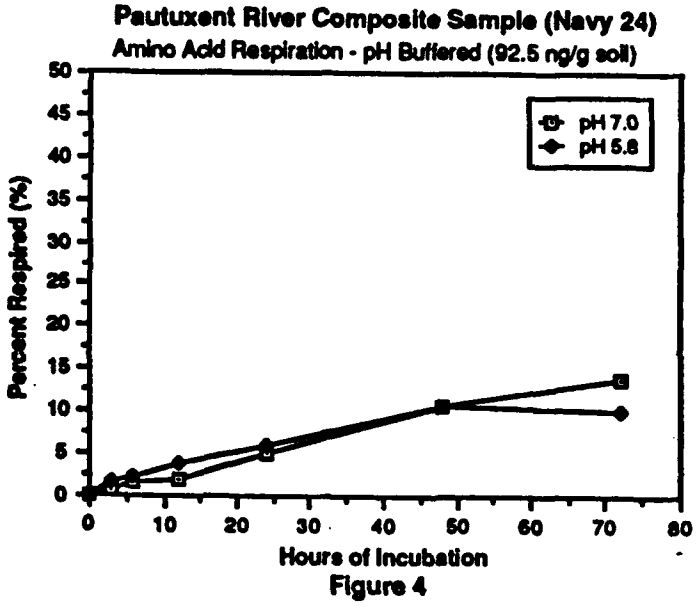

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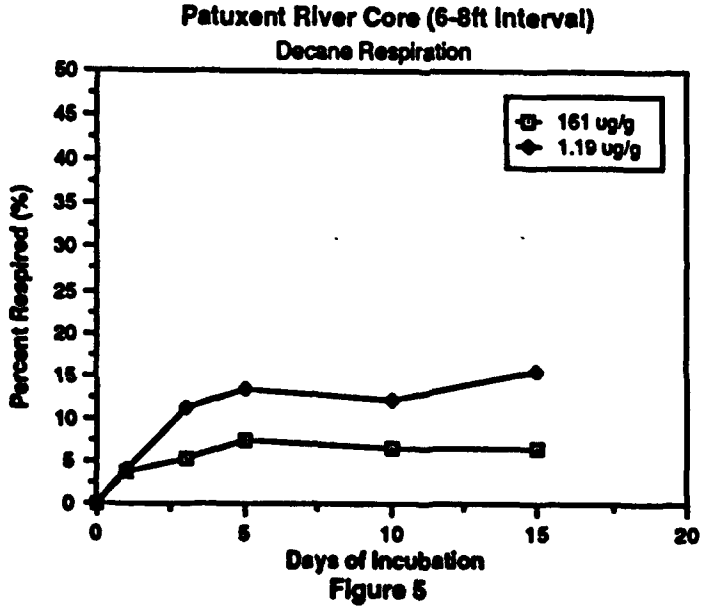

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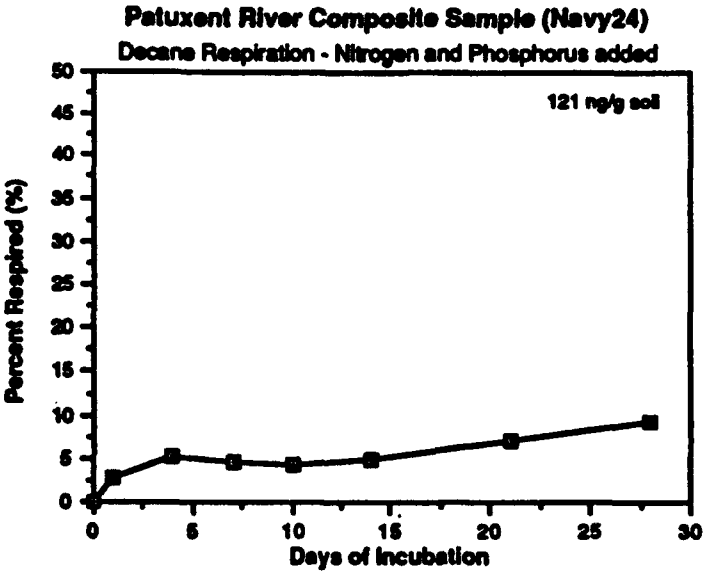

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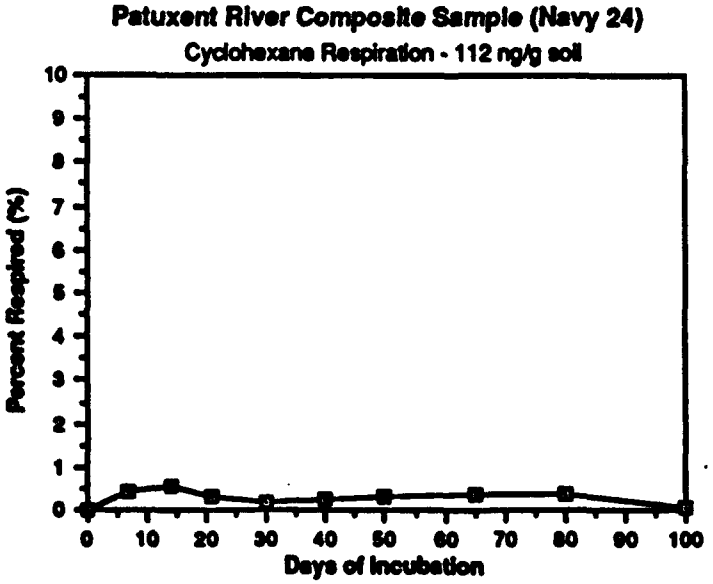

Figure 9

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